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IMMUNO-NEUROLOGY

Summary Reports of

an NRP Work Session held July 23-24, 1964

and of

Recent Experimental Progress

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Work Session Attendees

S. Bogoch	Boston University School of Medicine, Boston, Mass.
R. K. Brown	Wayne State University School of Medicine, Detroit, Mich.
P. F. Davison	Massachusetts Institute of Technology, Cambridge, Mass.
F. Huneeus-Cox	Universidad de Chile, Vina del Mar, Chile
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B. D. Janković	University of Belgrade, Belgrade, Yugoslavia
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L. Levine	Brandeis University, Waltham, Mass.
R. B. Livingston*	National Institutes of Health, Bethesda, Md.
Lj. Mihailović	University of Belgrade, Belgrade, Yugoslavia
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N. R. Rose	State University of New York at Buffalo, Buffalo, N. Y.
A. L. Rubin	Cornell Medical Center, New York, N. Y.
F. O. Schmitt*	Neurosciences Research Program, Brookline, Mass.
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INTRODUCTION TO "IMMUNO-NEUROLOGY" AND WORK SESSION REPORT

A Summary Dated March 22, 1965

by

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In May, 1964, F. O. Schmitt introduced the term "immuno-neurology" at an NRP Work Session on Macromolecular Means of Information Storage and Readout in Biological Systems. In his presentation "Molecular and Ultrastructural Correlates of Function in Neurons, Neuronal Nets, and the Brain,"(1) Dr. Schmitt suggested that the unknown correlates between structure and function might be found by means of immunological techniques, especially in view of the remarkable discovery by Mihailović and Janković(2) that the in vivo electrical activity of cat brain was affected region-specifically by intraventricularly-introduced antisera to homologous regional antigen. Accordingly, to explore the potentialities of a combined experimental discipline, an "Immuno-Neurology" Work Session was held at the NRP Center on July 23-24, 1964, bringing together the immunologists and neurologists listed on page 2.

There are at least two aspects of immuno-neurology.(1) One aspect consists of possible relationships in recognition mechanisms between the engram of the nervous system and the antibody of the immune system. These analogies are being given attention in other past and planned NRP Work Sessions, as well as in three discussion papers already published in this Bulletin.(3a-c) The other aspect, the experimental use of antibody as an analytical tool for neurology, was the central topic of this Work Session. It has been applied at two structural levels -- those of brain region and of cell constituent -- as well as to function.

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* Work Session Chairman

RESULTS OF RECENT EXPERIMENTS

The pioneers at the brain-region and function levels, Drs. Mihailović and Janković, were present at the Work Session to describe their experiments, and their communication is given here in full (page 8). Although they term their results "preliminary," their experiments clearly open an exciting possibility, the possibility of a functional dissection of the nervous system.

Among the exemplars of work at the cell-constituent level were Drs. Bogoch and Rapport. Dr. Bogoch described his separation of cerebroproteins of human grey matter. By means of more quantitative methods for the extraction of brain proteins and more adequate separation techniques, Bogoch found a larger number of proteins than had previously been realized; and he was beginning comprehensive immunological experiments to further characterize and localize the proteins in the brain (see ref. 4).

Since the time of the Work Session, further advances have been made in immuno-neurological experiments on brain protein, and are included in this report: an NRP-initiated cooperative effort by Moore and Levine (p. 18), and experiments by Work Session Participants Rubin and Stenzel (p. 23).

Dr. Rapport is to be thanked for his presentation on the immunochemistry of lipids to a protein-oriented group. His latest finding was a galactocerebroside antigenic determinant in the myelin sheath of the central nervous system (see ref. 5).

DISCUSSION

Use of Antibody to Detect Structure

The neurologists were particularly interested in the possible mediation of neuronal circuitry by molecules at cellular junctions,(1) and the general question was asked of the immunologists how well antibodies could recognize and differentiate among possible neural mediator molecules. It is difficult at this time to relate recognition potential as indicated by reaction with antibody and recognition potential as indicated by neuronal firing, but nothing to exclude the possibility was brought out. The immunologists provided a number of examples where serological reactions to molecules were distinguishable whereas the molecules were otherwise physically and chemically indistinguishable.

Among the experimental points stressed were: 1) an antigen of lower than a few thousand molecular weight must be coupled to a protein to be immunogenic, and 2) the coupling of antibody to ferritin, an electron-dense compound, is a useful method for localizing the antigen cellularly at the electron microscope level.

Functional Effects of Reaction with Antibody

In the discussion of the experiments of Drs. Mihailović and Janković, it was generally agreed that the structural correlate of the functional effects would involve cell surfaces, since the antibody molecule will not penetrate most living cells (although antibody in conjunction with the complement system will mediate lysis of cells).

In general, the effect of the combination of antibody with biologically-active structures is probably not a direct blocking of the biological activity. According to the textbook immunological view of anti-enzyme - enzyme systems, the relatively large antibody attaches to areas on the surface of the enzyme and sterically blocks the route of the substrate to the enzyme active center. A good example of this, presented by Brown, was that antibody to ribonuclease markedly inhibits the breakdown of ribonucleic acid by the enzyme but causes minimal inhibition of activity toward the low molecular weight substrate cyclic cytidine phosphate(6); and reactions involving dimer of cytidylic acid are inhibited more by antibody than those with monomer.(7)

However, the generalization that the antibody has never been directed toward the enzyme active center may not be quite right, in view of the fact that these studies have been based on competitive or non-competitive inhibition studies. In all cases, the evidence that the antibody is not directed toward the enzymic center is the fact that one gets non-competitive inhibition of the enzyme. Since the antibody molecule is divalent, with two receptor sites, when reacting with an antigen it has the capacity to polymerize and aggregate. When this occurs, equilibrium conditions are very difficult to obtain; so that the fact that one gets non-competitive inhibition may not be sufficient grounds for saying that in no case has antibody been directed toward the enzyme active center. Another possibility, for enzymes which change conformation during biological activity, may be that the combination with antibody prevents the transient conformational change requisite for biological activity by "freezing" the original structure.

Furthermore, instances have been found[8] where reaction with antibody can enhance the biological activity of the antigen. Antibody combination with the pharmacologically-active polypeptide bradykinin, rather than inhibiting pharmacological activity, sustains it. Since this polypeptide normally has a transient existence, evidently antibody combines with the peptide such that it does not interfere with the effect of the peptide on its target cell, but does interfere with the destruction of the peptide by proteolytic enzymes. Thus antibody can work two ways; and it is quite conceivable that an antibody to neural material may not inhibit activity but may activate it, if the neural functional molecule normally has a transient existence.

CONCLUSION

That immuno-neurology is rapidly becoming a fruitful discipline of study is clear, both from the presentations made at the Work Session and from subsequent experimental progress (Rapport, J. Neurochem.; Bogoch, Nature; Mihailović and Janković, Levine and Moore, Rubin and Stenzel, this Bulletin). In addition, one may look forward to future advances influenced by the exchange of views at this Work Session, in which immunologists and neurologists presented to each other the problems as well as the potentialities in the cooperation of their respective disciplines.

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EFFECTS OF ANTI-CEREBRAL ANTIBODIES ON ELECTRICAL ACTIVITY AND BEHAVIOR

A Preliminary Communication dated July 23, 1964

by

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The following is a brief summary of work which has been carried on for several years in our laboratories in Belgrade. It is largely due to the devoted efforts of our collaborators Drs. B. Beleslin, I. Divac, K. Isaković, K. Mitrović, D. Milošević and D. Čupić. Many of the experiments are still in progress and should therefore be considered as merely indicative.

Destructive processes, known as auto-immune or auto-allergic diseases, which affect various tissues, are usually accompanied by the formation of circulating antibodies that react in vitro with antigens isolated from corresponding tissues. However, it has not been shown that these antibodies are capable of inducing lesions when passively transferred into a normal animal of the same or different species. The apparent exceptions are experimental glomerulonephritis and immuno-hematological diseases in which the antigens carried by the cells are "accessible" to the corresponding antibodies. In recent years, there has been an increasing interest in auto-immunity as a possible cause of a variety of diseases affecting the nervous system; experimental allergic encephalomyelitis has been considered to be the basic immunological model used for this type of study.

Investigations performed in several laboratories, in which anti-nervous tissue antibodies have been infused systemically into normal animals, have consistently failed to demonstrate specific lesions. These unsuccessful attempts

did not rule out the possibility that anti-nervous tissue antibodies could exert an in vivo effect. However, no information could be found concerning the physio-pathological events associated with an in vivo collision between anti-brain antibodies and the corresponding nervous tissue. Our first purpose was to study these events.

In addition, the significance of proteins and other high-molecular weight compounds in cellular physiology has been increasingly recognized. An appreciable body of data suggested that memory, particularly the stable stage of information storage, could in one way or another be related to macromolecular synthesis. The fascinating possibility that, by applying immunochemical techniques, one could perhaps approach the study of the role of macromolecular nervous-tissue compounds in electrogenesis and in processes underlying learning and behavior, and even approach their identification, was the second main reason which motivated this investigation. Bearing in mind the high complexity of the morphological, biochemical and functional organization of the brain, a series of preliminary immuno-electro-physiological experiments was undertaken to investigate the possible differential reactivity of various cerebral structures when exposed to the action of different brain region antisera.

The lipid and lipoprotein fractions of brain used in immunological investigations by various authors had been obtained from the total organ, or from the grey or white matter. No attempts had been made to demonstrate serological differences between lipid antigens extracted from a particular region of brain and those obtained from other regions. Therefore, our attempts were first directed toward study of the action of anti-lipid antibody.

The results of the reactivity of anti-cat brain sera with isologous brain lipids showed that none of the anti-cat brain sera differentiated among the lipids isolated from different regions of the central nervous system of the cat. Furthermore, the cross-reactivity of the anti-cat brain sera with the lipids obtained from heterologous brain (dog, ox, monkey and man) was very pronounced, so that practically no difference could be observed between the reactions of anti-cat brain sera with isologous and those with heterologous brain region lipids.

Nevertheless, the anti-caudate nucleus serum absorbed with the lipids from the caudate nucleus, temporal cortex, and cerebral white matter still showed some reactivity for caudate nucleus. Since the lipid residues used as antigen contained a certain amount of protein, the possibility could not be excluded that the remnant serological activity might have been due to some non-lipid components. For this reason, in all subsequent experiments, homogenates of the whole brain region tissues were used as antigens. The caudate nucleus was chosen as the object of our investigation for three reasons: (1) The finding cited above, that anti-caudate nucleus serum showed some remnant serological activity against caudate nucleus after absorption; (2) because it forms the wall of the lateral ventricle and therefore could be easily attacked by the intraventricular route, and thus brought into prolonged contact with antibody injected into the cerebrospinal fluid; (3) because histological investigations of others (Fleischhauer) showed that the subependymal layer of glial fibers is thinnest there, which it was hoped would promote the resorption of the injected material.

Electrodes were implanted in various parts of the brain. The animals were injected with normal and immune anti-caudate and anti-hippocampal rabbit gamma-globulin, introduced through a Feldberg cannula into the lateral ventricle, in order to avoid the blood-brain barrier. In order to keep the animal tolerant, three to four daily injections with an interinjection interval of sixty minutes were given for four successive days.

In control animals, no essential change in electrical activity of various cerebral structures investigated could be noticed. Records made in the course of normal gamma-globulin application, as well as records taken at intervals within the period of one month following the last injection, were found to be fairly comparable with the activity recorded prior to the gamma-globulin administration. However, in the experimental group of animals, intraventricular application of anti-caudate nucleus gamma-globulin was followed by pronounced modification in electrical activity. The evolution of electrographic disturbances seemed to pass through three consecutive stages. The first stage was characterized by the appearance of spikes and high-voltage sharp waves localized in the caudate nucleus. These transient irritative abnormalities became apparent on the third day of antibody administration, and disappeared two to three days

afterwards. The second, also transient, stage was characterized by a slight general accentuation of background activity and by sporadic appearance of diffuse abnormalities consisting of bursts of four to six per second high-voltage waves, in all leads. Such activity outlasted the disappearance of spikes and sharp waves in the caudate nucleus for several days. The third stage was characterized by gradual slowing down, progressive decrease in the amplitude, and almost complete disappearance of spontaneous electrical activity of the caudate nucleus within a month after antibody administration. Such an evolution of electrographic abnormalities was reflected in modification of potentials in the caudate nucleus evoked by acoustic stimuli, as well as in the change of activity propagated to the caudate nucleus during seizures induced by electrical stimulation of the hippocampus. It should be emphasized that no change in electrical activity of the caudate nucleus could be observed in animals treated with anti-hippocampus antibody.

In brief, the initial irritative phenomena, followed by a progressive decrease in electrical activity strictly confined to the caudate nucleus, strongly suggested that anti-caudate nucleus antibody has an affinity towards the homologous nervous tissue. Furthermore, these findings suggested the possibility of regional specificity of some high-molecular-weight substances present in morphologically and functionally differentiated parts of the central nervous system.

Pursuing this line of investigation and having in mind the well-known fact that, in general, an antigen-antibody reaction in vivo is associated with a release of histamine, acetylcholine, 5-hydroxytryptamine, and other biologically active substances, a series of experiments was undertaken to test: (1) if a similar phenomenon takes place when the brain is attacked by an intraventricularly injected anti-brain antibody, and (2) whether or not a differential reactivity of various brain structures to the action of an antibody produced against the tissue of a specific cerebral region, as suggested by previous experiments, could be ascertained, using as indices the variations in the histamine contents.

Results showed that, in contradistinction to the normal rabbit gamma-globulin, an immune anti-cerebral gamma-globulin injected into the lateral ventricle of cat, produces an increase of histamine-like substance in the brain. Furthermore,

they demonstrated an apparently differential reactivity of various cerebral structures to the action of an antibody produced against the tissue of a specific brain region. The predominant increase of histamine-like substance in the caudate nucleus and hippocampus following the intraventricular injection of anti-caudate and anti-hippocampal antibodies, respectively, proved to be statistically significant.

Since, in the reported experiments, injection of anti-caudate gamma-globulin was associated with profound behavioral changes, such as depressive and catatonic states, we thought it would be necessary to investigate these changes in an appropriately controlled experimental situation.

In 1956 Rosvold and Delgado found that, unlike visual discrimination, delayed alternation test performance of monkeys is markedly impaired both by electrical stimulation and electrocoagulation of the tissue in the head of the caudate nucleus.

On this ground it was logical to assume that if the electrographic and biochemical disturbances observed in our experiments reflect an alteration in the functional state, and possibly of the morphological state, of the caudate nucleus, an impairment of delayed alternation test performance without change in visual discrimination test performance should be expected to follow an intraventricular injection of anti-caudate nucleus antibody.

This study is still in progress, and what follows should be considered as preliminary, being based on an insufficient number of experiments to be considered conclusive.

Monkeys were trained to perform tasks of delayed alternation and visual discrimination. Antibodies were made against the caudate nucleus and the hippocampus, using monkey brain tissue, and were injected intraventricularly as previously described.

Control animals receiving normal gamma-globulin did above criterion (above 90%) on both tests through an entire month. Animals receiving anti-hippocampal gamma-globulin exhibited impairment in the delayed alternation test during the injection days but immediately recovered and performed above criterion for the remainder of the month, and were not impaired in the visual discrimination. However,

animals injected with anti-caudate gamma-globulin showed a profound decrease in the ability to perform the delayed alternation test; while one of them performed above criterion on the visual discrimination task, another showed slight impairment in this ability.

Unfortunately, many experiments had to be discarded because of infection and other interferences which prevented evaluation of otherwise similar results.

In order to bring the antibody in direct contact with the cerebral tissues, a series of preliminary experiments was performed. In these experiments, antibodies against various brain regions, as well as normal gamma-globulin, were injected intracerebrally by means of a microinfusion technique, at a speed slow enough to match the absorption rate of the brain, in order not to create a large lesion.

The existence of identical structures in the two sides of the brain permitted comparing the effects of simultaneously instilling solutions containing different gamma-globulin in the opposing structures.

A pilot experiment showed that intracerebral injection into the hippocampus of the corresponding antibody gives rise to localized irritative abnormalities developing into a long-lasting epileptiform discharge. On this ground, the hippocampus was used for studying the possible differential effects of antibodies produced against various brain regions. In the few experiments done so far, anti-hippocampal antibody injected into the hippocampus proper of one side of the brain induced the kind of discharge described above, while rabbit normal and immune anti-caudate globulins, injected simultaneously into the hippocampus of the other side, failed to induce changes other than a noticeable decrease of the amplitude of the spontaneous background activity.

In some of our intraventricularly injected animals, hydrocephalus of various degree, often unilateral, was found. However, no correlation with respect to either the substance injected or the injection site could be observed. In no animal injected with normal rabbit gamma-globulin, however, was hydrocephalus found to be associated with the electrographic abnormalities described.

Histological examination of the brains, using classical

staining procedures (Nissl layer, etc.), has not so far revealed any specific morphological change which would provide a basis for explaining the differential electrophysiological, biochemical, and behavioral findings reported above. Some subependymal and perivascular infiltration with mononuclear and some endothelial cells of varying degree, more marked on the cannulated side of the brain, was a common finding in animals killed one month following the intraventricular injection period, regardless of whether normal or immune gamma-globulin had been injected. Some quantitative differences, such as more apparent change in the caudate nucleus than in other periventricular brain regions following anti-caudate antibody administration, can not be fully evaluated as yet. Brains of animals sacrificed four or five days following the initial intraventricular injection of normal and immune gamma-globulins - i.e., at the time when most prominent electrographic and behavioral changes usually occur - are at present being investigated.

Although the results presented challenge discussion in many directions, only some aspects will be briefly commented upon - namely, those pertaining to problems implied in the suggestion that there might exist in the brain some regional macromolecular specificity. Despite the high degree of differentiation, various portions of the gray matter of the central nervous system can still be considered morphologically similar in that they are all composed of neurons, glia cells, unmyelinated and myelinated fibers, blood vessels, elements of the supposedly common ground substance, etc. Therefore, in view of the high specificity of serological reactions, so amply attested by Landsteiner, it can be assumed that the immune gamma-globulins, used in our experiments, contained a bulk of antibodies against a number of various components present in homogenates of given brain regions, including nonspecific ones common to the brain as a whole. Yet, as could be judged on the basis of experiments herein reported, given anti-regional gamma-globulin was still able to "recognize" the corresponding nervous structures. It could be inferred from these considerations, that the predominant electrical, biochemical and behavioral changes were related to the part of the brain attacked by the corresponding antibody. This indicates that in the antigen-antibody reaction are possibly involved primarily those elements (whatever they might be: neurons, glia, or both) that determine the antigenic specificity of the given nervous structure.

Although experimenting on the brain proved advantageous for studying the differential reactivity of various cerebral structures to the action of a given anti-brain region antibody, the enormous complexity of its morphological, biochemical, and functional organization greatly hampers the investigation of fundamental mechanisms underlying the immunologically induced changes in cerebral electrogenesis. In order to approach these mechanisms in a more direct manner and in a simpler biological system, a series of experiments has been undertaken to investigate electrical phenomena occurring in a single nerve fiber exposed to prolonged action of the corresponding antibody.

It was found that membrane potentials of lobster axons immersed in the normal rabbit gamma-globulin solution, like those in artificial sea water, could hold for more than ten or even fifteen hours. However, the electrical activity of axons immersed in the immune rabbit gamma-globulin solution could in no instance be recorded longer than six hours, with most of them dying within less than three hours after the microelectrode insertion. Blocking of the action potential was always preceded by an important decrease of the resting potential amounting, by the end of the experiment, to at least 15 to 25 or even 30 mV.

Characteristic changes in the action potential could also be observed. Following an initial increase in the amplitude associated in a few instances with repetitive firing (seen also in some of the control experiments), the action potential became gradually smaller and broader and sometimes notched, until it finally disappeared. Subsequent insertion of the electrode into a new, adjacent axon usually revealed a somewhat lower resting potential, but the action potential, although small (there was no overshoot) and deteriorated, could still be recorded for one or even two additional hours before it became completely blocked. It is our impression that in contradistinction to the control experiments, the progressive decrease of the resting potential and the deterioration of the action potential were the most remarkable and consistent observations.

The number of penetrations seemed to have some effect too. In axons reimpaled several times, the above described sequence of changes took place within a much shorter period of time - usually less than an hour. In experiments with axons in the artificial sea water and normal gamma-globulin

solution, multiple and prolonged impalement were not found to cause such an important damage to the axon. Since the changes in trans-surface membrane potentials indicated an important extrusion of potassium ions, a simple experiment was carried out to check this assumption. A bunch of lobster nerves, desheeted in exactly the same manner as was done for electrophysiological purposes, was immersed in tubes with a given volume of artificial sea water containing the same amount of normal and immune gamma-globulin as in the experiments described above. Twelve hours later, twice as great an increment of potassium was found in a solution containing immune gamma-globulin (0.044 mgr per 14 mgr of nerve tissue). It is worth emphasizing that no histamine could be detected in any of the investigated solutions. In keeping with the generally accepted hypothesis that the resting potential reflects the potassium permeability of the resting membrane, the results presented seem to indicate that the prolonged exposure of the cell to the action of the corresponding antibody might, among other events, give rise to the deterioration of the potassium permselective property of the membrane.

It has been demonstrated by Huneeus-Cox that seven antigenic components could be resolved in the axoplasm of the giant axon of Dosidicus gigas. In view of his recent preliminary findings (unpublished) that the perfusion of the axon with anti-axoplasm antibody was found to be associated with alterations in electrical activity quite similar to those described herein, it is not unlikely that the much more striking changes that occurred following multiple impalements as observed in our experiments might have been due to the facilitated penetration through the injured membrane of some anti-axonal components contained in our immune gamma-globulin, which might indicate the dependence of EMF of the membrane upon some intra-axonal macromolecules too.

The experiments reported leave little if any doubt as to the observation that, in general, anti-cerebral antibody can cause profound alteration in electrical activity and behavior. This in itself provides a sound basis for a number of further experiments pertaining to the physiopathology of auto-immune processes and the underlying mechanisms.

With regard to the possibility that there might exist in the brain some degree of regional specificity of macromolecular substances, as suggested by our initial experiment,

results obtained so far are encouraging but not yet conclusive. Many more experiments are needed before any definite conclusion can be made. Outlined experiments will provide, it is hoped, an adequate framework for resolving many complex questions implied by the above-mentioned hypothesis.

Acknowledgement

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STRUCTURAL RELATEDNESS OF A VERTEBRATE BRAIN ACIDIC PROTEIN AS MEASURED IMMUNOCHEMICALLY

A Research Report dated March 10, 1965

by

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A protein unique to nervous tissue was recently discovered in a number of vertebrate species (rat, rabbit, guinea pig, chicken, catfish, alligator, beef, monkey, and human), and the protein from beef brain was isolated and purified (Moore and MacGregor, 1965; Moore, 1965). The protein, an unusually acidic component of the brain-extract soluble fraction, was found in roughly equal concentrations in grey and white matter and spinal cord. In September, 1964, a conference was held at the NRP Center attended by F. O. Schmitt, B. W. Moore, L. Levine, P. F. Davison and B. Smith, in order to initiate cooperative immunological studies on the protein to elucidate its neurophysiological role. This communication is a report of preliminary experimental results on the antigenicity of the protein. Using antibodies to this acidic protein, it has been shown that, in contrast to most vertebrate proteins, the structure of the acidic protein exhibits little evolutionary variation.

Since antibodies to the purified beef brain acidic protein (BBAP) could not be obtained by direct immunization

(Moore, unpublished), a procedure was applied to make the BBAP immunogenic by complexing it with methylated bovine serum albumin (MBSA), a method which had been used successfully to obtain antibodies to negatively charged molecules such as DNA and polysaccharides (Plescia, et al., 1964) and polyribonucleotides (Levine, et al., 1965). Antibodies to BBAP antigen were successfully produced using MBSA - BBAP complex as the immunogen, and the antiserum was shown to be immunochemically homogeneous in double diffusion tests in agar with crude beef brain extract and purified BBAP as antigens.

In double diffusion experiments with this antiserum, a precipitating band of identity was found with brain extracts of several species. The complement (C') fixation curves obtained with antiserum to the purified BBAP and brain extracts of several animals are shown in Figure 1, at antiserum dilutions of 1/4000 (left) and 1/2000 (right). Since the absolute content of acidic protein in each brain extract is not known, the maximum fixation for each extract is plotted at the point of maximum fixation for purified BBAP, 0.2 μg. It can be seen that while there are quantitative differences in the brain-extract antigens among different species, these differences are extremely small when compared to similar data obtained with other protein immune systems. For example, the data of Tashjian, et al., (1965) show much greater differences among the primate growth hormones when tested with antisera to human growth hormones (Figure 2), squirrel monkey growth hormone requiring three times more antiserum for reaction with anti human growth hormone serum than for the homologous reaction. With bovine, ovine, and porcine growth hormones, 15 times as much anti human growth hormone serum is required before C' fixation is detectable. Such variations in serological activity associated with evolutionary differences have also been seen with hemoglobins, lactic dehydrogenases, triosephosphate dehydrogenases, glutamic dehydrogenases, and aldolases (Wilson, et al., 1964) and with many serum proteins (Goodman, 1962). The lack of species differences in this brain acidic protein, therefore, is striking. Only one other protein is known to resemble the brain acidic protein in this respect -- lens protein.

The serological activity of the rabbit brain extract is of further interest. Although the serum of the immunized rabbit contains antibodies directed toward the animal's own brain protein, the rabbit has shown no signs of illness. Either the blood - brain barrier is sufficient to shield the

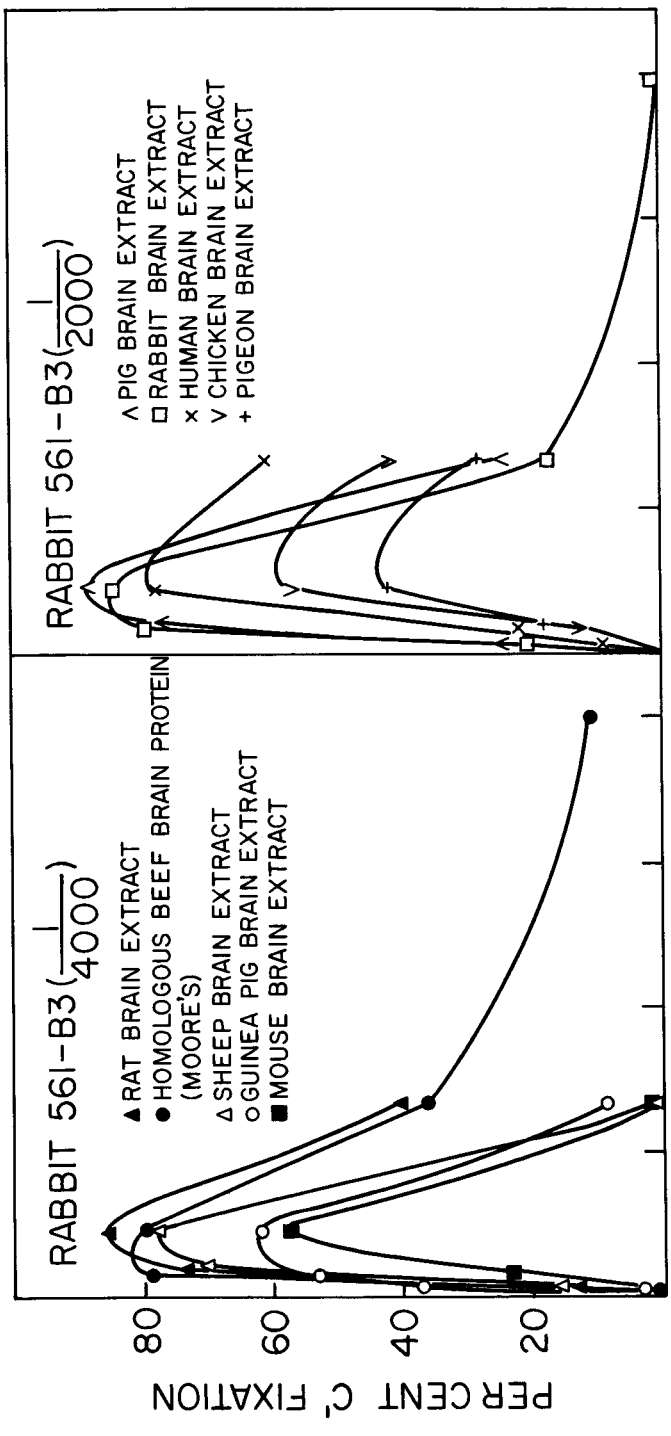

Figure 1. Complement fixation with various brain extracts and anti-BBAP.

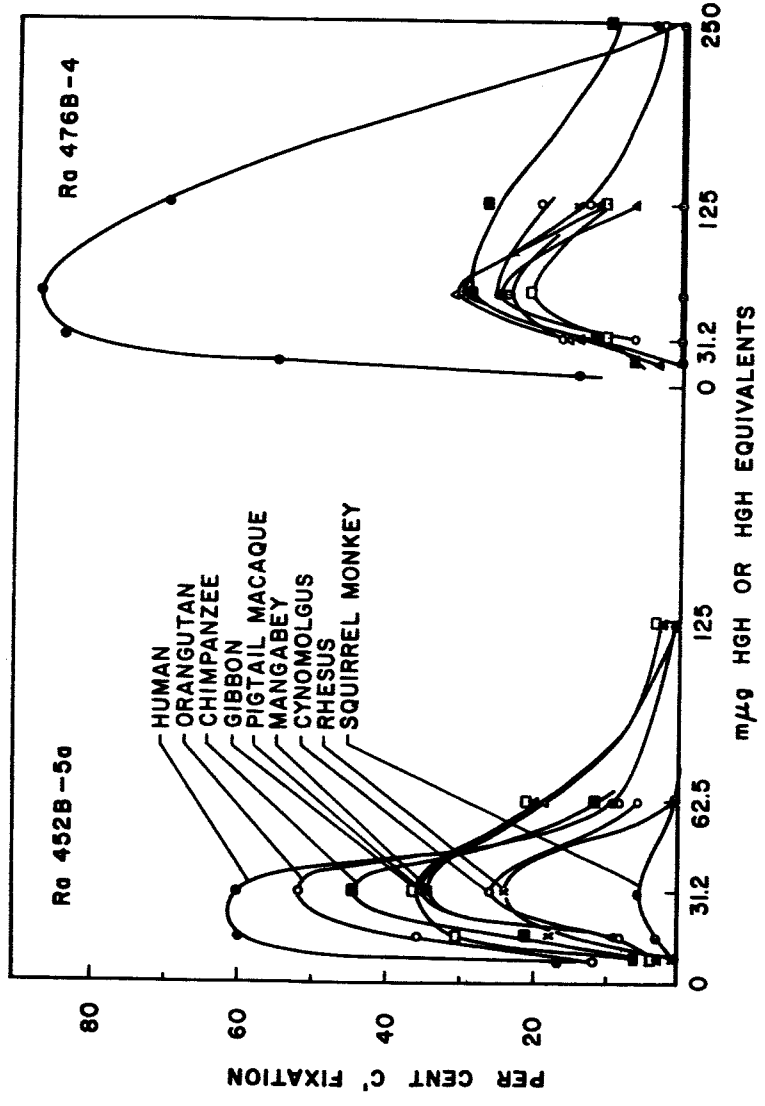

Figure 2. Complement fixation with purified human growth hormone and pituitary extracts of various primates and anti human growth hormone. Fixation curves on the right were obtained with antiserum to human growth hormone of low prolactin content. Curves on the left were obtained with antiserum to human growth hormone of high prolactin content. (From Tashjian, et al., 1965).

brain from effects of the antibody, or the acidic protein is located intracellularly -- a site which can not be reached by the circulating antibody. The cellular location of the protein is currently under study in experiments using labelled antibody.

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CELL-FREE SYNTHESIS OF A SPECIFIC BRAIN PROTEIN*

A Research Report dated March 1, 1965

by

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Neurons possess a highly developed ribosome-rich endoplasmic reticulum, similar to that found in exocrine gland cells. This morphologic characteristic, as well as results of metabolic studies on whole brain and brain slices, indicates that neurons are among the most active of protein synthesizing cells. Several proteins from nerve tissue have been isolated and characterized. An acidic protein has been purified from squid axoplasm.[1-3] Bogoch has separated a number of protein fractions from human grey matter.[4] Moore has extracted a protein from several types of vertebrate brain, present in no other tissue, and also highly acidic.[5] Antibody to this protein shows cross reaction with similar material isolated from the brains of several species.[6] The functional significance of this protein and of the high rate of protein synthesis in neurons is unknown.

We have investigated the nature of protein synthesized in cell-free systems obtained from rabbit brains. Cell-free systems capable of incorporating amino acids into trichloroacetic acid (TCA)-insoluble material have been derived from plant, bacterial and animal cells. The essential ingredients of these systems are quite similar and consist of ribosomes (with attached informational or messenger RNA), transfer RNA

* This summary is based on a report presented at the NRP Ninth Stated Meeting, February 5, 1965.

and transfer enzymes, ATP, and an ATP regenerating system, GTP, and an isotopically labelled amino acid. The amino acid becomes incorporated into TCA-precipitable material, presumably protein, when the reaction mixture is incubated at 37° C.

Brain microsomes are active in protein synthesis[7-11] and some attempts have been made to characterize the protein formed.[12] We will describe the preparation and characteristics of brain ribosomal and microsomal cell-free systems and some properties of the soluble protein product.

The grey matter of rabbit brain was minced and homogenized; and the cell debris, nuclei, myelin, and mitochondria were removed by centrifugation. The post-mitochondrial supernate was either centrifuged directly to obtain a microsomal and soluble fraction, or it was treated with desoxycholate to obtain a ribosomal fraction. Activating enzymes and sRNA were prepared from the soluble fraction by precipitation at pH 5. Sucrose density gradient centrifugation on desoxycholate-treated post-mitochondrial fractions showed the presence of polysomes.

When complete reaction mixtures were incubated at 37°, radioactively-labelled leucine was incorporated into TCA-insoluble material linearly with time for 20-30 minutes; the incorporation was dependent on sources of ATP and pH 5 enzymes and was inhibited by ribonuclease and puromycin. Centrifugation showed that 10-15 per cent of the TCA-insoluble counts were present in the soluble fraction, i.e., were released from the ribosomes. This newly synthesized "protein" was separated from free leucine by chromatography on Sephadex, and this purified product was then used for electrophoresis, chromatography, and immune coprecipitation.

The bulk of this material had about the same electrophoretic mobility as albumin (isoelectric point 4.9) when separated in a Hannig free flow electrophoresis apparatus. Chromatography on DEAE cellulose revealed the radioactive material to be strongly absorbed, and elution was only accomplished after washing the column with 0.1 N HCl or NaOH. Immune coprecipitation was carried out by incubating a mixture of cell-free synthesized material, Moore's "S100" protein, and antibody directed against the S100 protein. Other incubation mixtures containing the radioactive protein and unrelated antigens and their antibodies were used as controls. Protein synthesized in a rabbit reticulocyte cell-free system was also incubated with S100 protein and its antibody as a further control of specificity. Immune precipitates were washed in cold

saline, and the number of counts coming down with immune precipitates was compared with the total number of TCA-precipitable counts present. Results to date indicate that 10-15 per cent of the soluble protein formed in these brain cell-free systems was specifically precipitable by antibody directed against the S100 protein.

Work is in progress[13] to study the protein formed in brain cell cultures as well as in cell-free systems. The effect of various agents, including proteins important in nerve and their antibodies, on brain cell growth and metabolism, and subcellular protein synthesis will be investigated. In vitro models such as these may provide the means for correlations to be made between subcellular activities, cell metabolism, nerve impulse propogation and synaptic function.

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THE ORGANIZATION OF MEMORY FUNCTIONS IN THE VERTEBRATE NERVOUS SYSTEM

A Discussion Paper

by

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CONTENTS

INTRODUCTION

Memory in nervous systems might be thought to result from functionally induced changes in the receptive fields of neurons. Such changes might arise from: 1) localized alterations in the chemical and electrical properties of postsynaptic membrane; 2) local alterations in the gross morphology of (either or both) post- and presynaptic structures, such as enlargement of boutons terminaux, or growth of gemmuli or dendritic "spinelets" (Van der Loos, 1964); or 3) the stimulated synthesis of protein macromolecules by which information might be encoded, transmitted across the synapse, and "recognized" postsynaptically (Schmitt, 1964; Silverstein, 1963).

Apropos of the third possibility, Horridge (1964) discusses the role of convergence (on a single neuron) in establishing conditioned responses: "One may quickly see how such a mechanism could work by supposing that arriving impulses which represent the conditioned stimulus have a lasting effect on the interneuron and modify its response only if impressed by the immediately subsequent arrival of impulses representing the unconditioned stimulus.... The widely valid finding that to be effective the US /unconditioned stimulus/ must not precede the CS /conditioned stimulus/ shows that in some way the neurons which carry the US can be distinguished from those which carry the various possible CS's. Mere convergence is not sufficient for an association to be formed. In view of its stability, the author is inclined to suppose that the feature distinguishing the CS and US is chemical...."

Unconditioned stimuli, in other words, correspond to a class of inputs which tend to give preferred status to any immediately preceding input, and to be without effect on any immediately succeeding one. (The ground for this last conclusion is the apparent impossibility of "backward conditioning"; Woodworth and Schlosberg, 1954). Almost by definition, any depolarizing or hyperpolarizing input to a neuron transiently alters the structure of postsynaptic membrane at the input site(s). If the half-life of these ultrastructural changes is sufficiently long, it might only be necessary to assume that subsequent inputs, equivalent to the US, issue a "freeze" order, or stimulate the neuron to stabilize existing membrane-structure by a) conserving certain features of the local structural changes just mentioned and

b) reducing the probability of other such changes, through general rises in thresholds. Memory formation in a single unit would thus consist in a generalized desensitization minimally affecting those postsynaptic sites which were most active at the time of arrival of the stimulus that induced the desensitization. This view implies that a neuron "remembers" or "recognizes" certain inputs, in part because of a diminished probability of responding to others; that is, learning, as suggested by Sharpless (1964), may in some respects be the converse of denervation sensitization.

The "order" bringing about such changes might involve a presynaptically released transmitter and the RNA apparatus of the responding unit (Mahler and Moore, 1964. See accompanying note). No specific chemical coding (one macromolecular species of specific structure per "bit" stored) would be required. A class of chemical transmitters perhaps rather few in number and maximally concentrated in subcortical "motivational" systems, might suffice. (It is interesting that a similar segregation of learning or "mapping" and "motivational" or consolidative, functions appears to have occurred in the octopus brain, which is in other ways very unlike the vertebrate and represents a line of evolutionary development divergent from the mammalian over many millions of years. Young, 1964). The clinical data of Scoville and Milner, (1955,1957), suggest that in man, it is the septal-hippocampal system which issues orders, e.g., to temporal-entorhinal cortex, to "fix" a given input. Apropos of the transmitter mechanism just discussed, it is of interest that the rhinencephalic motivational systems in mammals represent a phylogenetically old division of the forebrain whose chief diencephalic relay is the hypothalamus and whose primordial function seems to have been olfactory. (Concerning the anatomy of the rhinencephalon see Pribram and Kruger, 1954, and Papez, 1958. Concerning the conversion of parts of it to "nonspecific" or motivational functions, see Herrick, 1948).

Neurosecretory cells may exist in rhinencephalic as well as diencephalic parts of this subcortical system, e.g., in the septal Islands of Calleja (MacLean, 1959). Knowles (1964) concludes from comparative studies that diffuse neuropil and a high proportion of neurosecretory cells may be characteristic of more primitive nervous systems (e.g., in coelenterates, platyhelminthes and annelids. See also his analysis of neural and neurosecretory elements in the crustacean eyestalk, op. cit., p-12). It is thus barely possible that learning in

mammalian neocortex is contingent upon the activity of subcortical structures because these, in contrast to neocortex, represent a system in which (in Horridge's phrase) "chemical addressing" has not been as largely supplanted by "addressing" dependent upon circuitry or cytoarchitecture.

The hypothesis of memory functions adopted here is thus partly "structural" and partly "macromolecular". It seems to the writer that present evidence favors such a composite theory. Proponents of the macromolecular theory have argued that the absence of covalent cross-linkages in neuronal unit membrane may rule out structural alterations as a possible basis for long-term memory (Lehninger, 1964). However, the type of membrane structure proposed by Warner, and subsequently modified by Hechter (1964), might obviate that objection.

The work of Furshpan and Furukawa (1963) and of Diamond (1963) on the Mauthner cell makes it clear that stable regional differences in the properties of membrane can exist, at least in some neurons. Dethier's work on taste receptors in the fly suggests but does not conclusively show, that a given dendrite may have distinct receptor sites, e.g., for fructose and glucose (see Weiss, 1965). Like the site-specific responses of the Mauthner cell (to GABA), these regional differences, if actual, may be determined by heredity and resistant to later change, the same applying to the differentially responsive "patches" of postsynaptic membrane postulated by Terzuolo and Bullock (crayfish stretch receptor. See Bullock, 1959). To serve as a basis for memory such a patchwork structure should be alterable; and such functionally induced changes should show gradations of reversibility, corresponding to long- and short-term forms of memory.

A structural alteration hypothesis is not inconsistent with the data of Hydén et al. (1963) and Morrell (1961). Their evidence suggests that during "driving" of a unit (perhaps crudely equivalent to psychological "reinforcement") the RNA apparatus of the cell is mobilized, so as perhaps to produce the types of general and localized membrane change just discussed. Once established, e.g., in successive subsystems in one or more circuits from central stations to the final effector paths, such changes would in effect constitute a new, or functionally established pathway, with some or most of the performance-characteristics of innately determined ones. This view of memory fits well with the fact that while some

observed consolidation times are relatively long (Abt and Essman, 1961; Flexner, Flexner and Stellar, 1963. However, see Chorover, 1964 and accompanying note), suggesting slow RNA-mediated structural changes, retrieval of memory-data may occur on a scale of tens or hundreds of milliseconds (see e.g., Adey et al. 1963), suggesting activation of neuronal circuits only.

The position tentatively taken here, then, is that memory may result from the alteration of central pathways in consequence, chiefly, of site-specific alterations in the postsynaptic membrane of neurons; possibly accompanied by general threshold changes the reverse of those occurring in deafferentation or sensory deprivation (re the last see e.g., Melzack, 1962). The role of certain neurotransmitters, notably norepinephrine and serotonin, may be to stabilize such changes in the properties of neuronal receptor membrane—which is to say, these substances may not be transmitters in the same sense that acetylcholine is.

The foregoing might be termed the residual differentiation hypothesis of memory. For the discussion which follows, the essential point is not the mechanism by which such differentiative changes may be induced. It is 1) that they may occur, and 2) that they may be temporally stable or irreversible in certain neuronal populations and not in others. The object of this paper is principally to show that two such populations, distinguishable by clear anatomic and histochemical criteria, may exist in the vertebrate central nervous system.

FUNDAMENTAL CONSIDERATIONS RELATING TO THE ORGANIZATION OF BEHAVIOR

Sperry has said that the whole of the central nervous system must be regarded as effector. Stated another way, central nervous processes are essentially adaptive, and adaptation is essentially a matter of actions. So long as the conditioned stimuli triggering a given response (autonomic and skeletal motor components included) are sufficient to predict <u>most</u> of the situations in which it is likely to be needed, they will serve. When allowance is made for the factor of "latent learning" (Woodworth and Schlosberg, 1954), Lashley's principle of economy in the recall of sensory detail (1938) may still hold. Conditioned responses may thus resemble innate responses,

in that the conditioned stimuli which activate them, like the "sign stimuli" which release unlearned behavior (Tinbergen, 1951), tend to be few and simple.

The question is: how is this economy accomplished? This problem is related to another one which nervous systems, in order to succeed adaptively, must solve. Namely, given nearly continuous inputs representing (in Sherrington's phrase) a "million-sided environment", how does the brain select certain sense-data adequate for the guidance of behavior, and then transform these into practicable instructions to the effector systems? The problem of learning is, then: by what mechanism(s) do those sense-data which have most successfully guided behavior become items of recall, or part of a complex including the learned behavior itself? Certain tentative answers to these questions are suggested by the architectural plan of the vertebrate nervous system. Two features of that plan to which I would particularly direct attention are: 1) the prevalence of feedback loops, and 2) the pattern of convergence and redivergence frequently encountered in traversing one subsystem and entering the next along a given pathway.

CENTRAL SERVO-MECHANISMS

To consider these two points in order, it is clear that feedback is not only general in nervous systems, but also occurs on several scales from the large intersystemic to the very small, on which path length is measured in tens or hundreds of microns. The latter is illustrated by the organization of neocortex into columns, or "vertical internuncial chains" (Lorente de Nó, 1949). Feedback loops on this size order evidently figure in "lateral" or "surround" inhibition, e.g., in the retina, in the olfactory bulb (Yamamoto et al. 1963), or in the skin and somatosensory cortex (Mountcastle, 1957; Mountcastle and Powell, 1959). Present evidence suggests that such feedback is predominantly, but not exclusively, negative (inhibitory), and there appear to be strong theoretical arguments as to why that should be the case (see for example, Arbib, 1964). One might conveniently distinguish two classes of central servo-mechanisms.

The first involves playback from major effector divisions of the nervous system, and may have the effect of limiting central outflows to a relatively small number of preferred (= concurrently most active) pathways. Brooks (1963) has demonstrated feedback inhibition in somatosensory cortex

by antidromic stimulation of the medullary pyramid. He was able to show that the latter, when paired with peripheral stimulation, reduced the peripheral fields of postcruciate units. He described this as a possible mechanism for increasing "contrast". (See also Eccles, J.C., 1963, for a brief discussion of how both post- and presynaptic inhibition might produce this result.)

Depending upon the timing of stimulation of the anterior lobe of the cerebellum relative to click-stimuli, Snider and Sato (1958) obtained depression or, at longer intervals, enhancement of evoked cortical potentials in areas AI and AII in the cat. Moruzzi, also stimulating in the cat cerebellum, obtained apparent suppression of a motivational state (rage) with parallel pupillary changes and post-stimulatory rebound (see Ingram, 1960). Recently, Starr (1964) has shown that movement concurrent with auditory stimulation depresses evoked cortical potentials (cat), this result being independent of the middle ear musculature. If, as seems justified on several grounds, one regards the midbrain reticular formation as an effector system relative to neocortex -- i.e., one lying synaptically closer to the final effector pathways -- then reticulocortical input might properly be included in this class of central servo-mechanisms. Here both positive and negative feedback seem to be involved, though Purpura (1959) has suggested, and Evarts (1962) has given evidence that with rises in non-specific input, the proportion of inhibitory to excitatory effects in neocortex may increase. That such input does in fact heighten "contrast" -- or in this instance, the resolving power of the visual system -- is indicated by a study by Fuster (1961), as well as by the fact that stimulation of the midbrain reticular formation (RF) can raise flicker-fusion frequencies (Lindsley, 1958). A second important type of feedback, overlapping in its origins with the foregoing, is that from central stations to the sensory periphery. The existence of centrifugal fibers ending on amacrine cells of the retina was inferred by Cajal (1909; see also Estable, 1961). A topically organized return pathway to the retina from the avian isthmo-optic nucleus has lately been described by McGill (1964). Reticular fibers reportedly distribute to the olfactory bulb of the rabbit (Green, 1961), e.g., via the anterior commissure, and may figure in the inhibition of mitral cell activity obtained by Yamamoto et al., (1963). Dewson (1963) reports that, with bilateral ablation of insulotemporal cortex in the cat, disinhibition of evoked potentials occurred at lower stations in the auditory relay, e.g., the cochlear nuclei. One might also mention the work of Hernández-Peón on apparent reticular "gating" of inputs in the gracile and

cuneate nuclei (1961) or in the visual pathway of the cat (1956).

Whereas feedback mechanisms of the first class may act to limit and give focus to central outflows to the effector systems, those of the second may serve to establish foci of perceptual attention. In establishing the latter, central mechanisms corresponding either to "innate releasers" (Tinbergen, 1951) or to conditioned stimuli (i.e., memories) may be important, in that central-peripheral playback via these may result in selection of certain inputs. A model of this kind, involving playback from a central "organizer" to a "comparator" or primary receiving system, was used by D.M. MacKay (1963) to derive a generalized form of the Weber-Fechner law. The possible relation of some of these feedback mechanisms to learning is discussed in later parts of this paper.

STRUCTURAL COMMON DENOMINATORS IN CENTRAL NERVOUS SUBSYSTEMS

We turn now to a consideration of the pattern of convergence and redivergence often found along a given neural pathway. Within subsystems at the sensory and effector periphery (retina, olfactory bulb, ventral horn) there is a high order of convergence (on retinal ganglion cells, mitral cells and motoneurons). In the case of sensory subsystems, redivergence occurs at the next one to two synapses. For example, while optic nerve fibers are approximately 1:1 with lateral geniculate units (monkey; Glees, 1961) the distribution of terminal fibers is approximately 1:5; and the population of striate cortical cells reached by units of LG is very much larger, even if one only take into account those striate units having "simple" retinal fields (Hubel and Wiesel, 1962).

In respect to structure, the neocortex has a qualified resemblance to peripheral subsystems in that input to any given cortical "vertical column" (from the relay nuclei or dorsomedialis or lateralis posterior) tends to be concentrated in "granular" (parvocellular) layers, notably IV and lower III (Lorente de Nó, 1949; Nauta and Whitlock, 1953). On cyto-architectural grounds Lorente de Nó made a major division of neocortex into the external lamina (layers I-IV) and the internal (layers V-VI). The latter was reported by him to receive relatively few direct inputs from the relay and association nuclei, whereas layer V in particular does receive a heavy concentration of collaterals of axons of units in the overlying

layers. By contrast, layer IV receives few if any such collaterals. The internal lamina also differs from the external in that it is apparently the chief source of the callosal and long association fiber-systems. With some exceptions (e.g., layer III of Brosa's area; see Bailey and von Bonin, 1951), arrays of large pyramids in neocortex are chiefly found in layer V. The density of these arrays and the mean size of their components tend to increase as one moves from koniose (prime receiving) cortex (Brodmann areas 3-1-2, 17, and 41-42) to generalized eulaminate (association) cortex, and frontal agranular (motor) cortex (Bailey and von Bonin, 1951). The reverse trend is shown by the "granule" cell layers, which are most prominent in the external lamina of prime receiving areas, and become greatly reduced in motor cortex (see Fig. 1). It would thus appear that the external and internal laminae bear a rough resemblance to the small-celled input and large-celled output divisions of subsystems lying closer to the periphery. Moreover, as one might expect, the external lamina is relatively the more developed in prime receiving areas, and the internal in sectors such as the motor and premotor.

In the cerebellum, major inputs are to the granule cell layer, while outputs are by way of the Purkinje cells. Important reticulospinal paths originate in medial magnocellular portions of the brainstem RF (Papez, 1929) whereas a laterally lying division of this same system, having both small- and large-celled divisions, receives spinal projections and has major (reciprocal) connections with the cerebellum (Crosby, Humphrey and Lauer, 1962). Finally, in the ventral horn, output units tend to be large (e.g., 70μ in diameter, or larger than the largest Betz cells in motor area 4), while many of the inputs to these from higher levels appear to pass via a small-celled internuncial system (Ruch, 1951; Kuypers et al., 1960).

The structural plan of the CNS can then be summarized as follows. From the sensory periphery, inputs are relayed over branched (i.e., parallel) pathways each consisting of n subsystems in series. In each of the latter, small-celled aggregates tend to be concentrated on the input side, and large-celled aggregates on the output side. Over several synapses, beginning at the receptors, there tends to be an increase in the cell-populations of successive subsystems and in the proportion of "granules" in each. Over the remaining synapses to the final effector paths, the reverse trend is observable. Both the proportion and the absolute size of output units begin to increase, while system cell-populations begin to decrease.

Fig. 1. STRUCTURE OF THE HUMAN CEREBRAL CORTEX

A. Principal Types of Neuron Found in the Cerebral Cortex

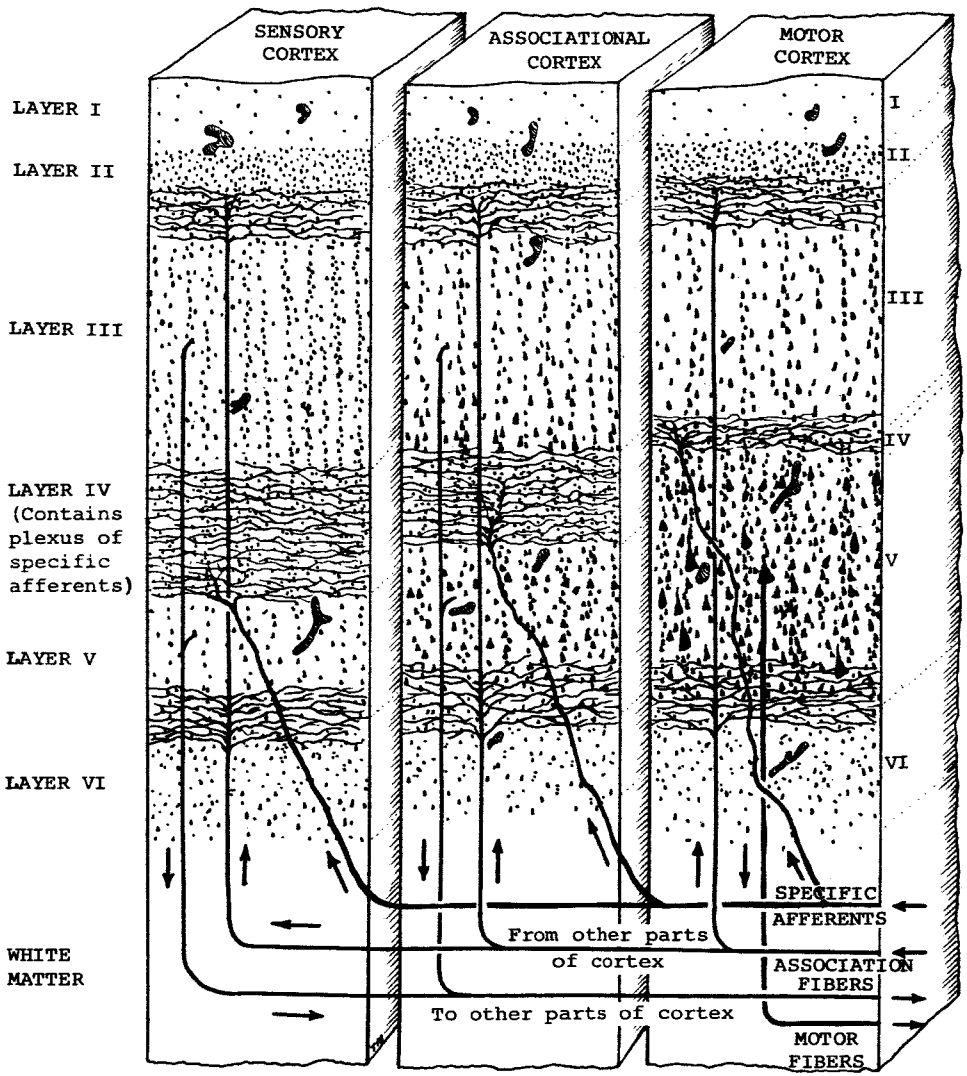
	DESCRIPTION		DESCRIPTION
	Pyramidal cell with unbranched axon to white matter		Stellate cell with axon distributed within the dendritic field of the cell
	Pyramidal cell with branched axon to white matter		Stellate cell with axon to white matter
	Pyramidal cell with branched axon to white matter and recurrent collaterals		Stellate cell with axon to outermost cortical zone
	Pyramidal cell with axon forming recurrent collaterals and branches only		Spindle cell with axon ending in Layer VI and apical dendrite to Layer V

Adapted from D. A. Sholl, The Organization of the Cerebral Cortex (N.Y.:Wiley, 1956).

Fig. 1A (above) shows the principal cortical cell-types as schematized by Sholl. It includes, in addition, the layer VI spindle, of which Lorente de Nó describes three variants: the "long" spindle with dendritic branches in VI and I, the "medium" spindle with apical dendrites ramifying in IV (see page 38), and the "short" spindle whose dendritic shaft ends in V (Lorente de Nó, loc.cit.).

In Fig. 1B (right), the bodies of smaller cortical cells, or "granules," are indicated by dots, and those of pyramids by roughly triangular figures. It is intended chiefly to show the changes in cellular composition encountered in six-layered cortex as one moves from prime receiving (sensory cortex) to effector (motor cortex) areas. Note that association cortex is in some respects transitional between the two; and that in motor cortex, layer V is greatly expanded and IV extremely thin.

B. Cytoarchitecture of Sensory, Associational, and Motor Cortex



Adapted from F. H. Netter, Nervous System (Ciba, 1962).

The former trend reaches a maximum in prime receiving areas of neocortex. The latter trend then begins with the appearance of large-celled bands in layers IIIc and V of generalized eulaminate (association) cortex, continues, as described above, in dysgranular and agranular (motor and premotor) sectors, and reaches a maximum in the ventral horn.

In neocortex the plan just outlined is modified in certain important respects. Whereas in the retina, the olfactory bulb, the cerebellum and the ventral horn, the entire output apparently passes via magnocellular assemblies, the same is evidently not true of "vertical columns", e.g., in motor area 4. It is well known that the Betz cells of layer V in motor cortex account for only about 2% of the pyramidal tract fibers in man. In the cat, there is clear evidence that pyramidal tract units, in both pre-and postcruciate cortex, are not confined to the internal lamina. See for instance, Towe, Patton and Kennedy, 1963. Of the 623 units tested by these authors 75% were found at depths of 800-1800μ. The extreme range over which some pyramidal tract units could be located was from 450-2260μ. The foregoing suggests a probabilistic rather than a strict distribution of output units in cortical columns. In fact, Lorente de Nó (1949) states that while most of the pyramids of layer V in mouse parietal cortex have axons which enter the white matter, and most of the cells of layers I-IV do not, there are exceptions, e.g., the "large star pyramids" of the external lamina (loc.cit., p.301).

The same principle may apply on the input side of cortical columns. In contrast to the retina, where apparently no input can bypass the bipolar cell layer, there appear to be deep early activated units in motor and somatosensory cortex, suggesting bypassing of layers I-IV, or direct activation of units of the internal lamina (Amassian, 1964; Towe, Patton and Kennedy, 1963). If one calculates the percentages of "simple field" cells found by Hubel and Wiesel in layers II-VI of cat striate cortex (see Fair, 1963), one obtains peaks conforming to the distribution of specific thalamic afferents reported by Lorente de Nó, but also comparable values, especially for layer VI of the internal lamina. It might be noted that in general, monosynaptic activation of deep units could be due to cells such as the medium pyramids of layer V or the medium spindles of VI, both of which have dendritic shafts which ramify and end in the plexus of specific afferents of layer IV, (Lorente de Nó, 1949).

It would appear, then, that in comparison to the olfactory bulb or the retina, the neocortex is a "leaky" system. In that not all columnar outputs need pass through magnocellular arrays (whose simplifying functions are discussed below), and that not all inputs need be "processed", in small-celled aggregates, e.g., of the external lamina, before relay elsewhere, the neocortex may gain over peripheral systems in functional flexibility. One result of this arrangement may be that a maximal diversity of commands is issued from this level to the final effector systems, with the consequence that the combinative capacity of the latter tends to be used to the full. As will be clear from the discussion below, this same arrangement may act partially to by-pass the cortical memory apparatus, and so prevent the behavior of the animal from becoming habit-ridden or overdetermined by its earlier experience.

FUNCTIONAL CORRELATES OF CENTRAL NERVOUS MORPHOLOGY

With due allowance for local variations, the structural plan just described may be sufficiently similar in all neuronal subsystems to justify the inference of a common modus operandi. The latter may be roughly as follows.

The function of small-celled arrays may be to "process" input to the system. In peripheral and central systems, the operations covered by that term presumably differ; but, in both, the element of selection will figure; and, in selection, the element of context will be most important. What is relayed centrally by one group of retinal units will depend in part upon what adjacent units are receiving. More generally, what happens to a given item of information will greatly depend upon what other items are introduced into the same system at the same time. The extent and sensitivity of this type of interdependence is presumably a function of the fineness of structure and the degree of cross-coupling in the system concerned. Hence, the probable importance of short-axoned cells to the "delicacy of function" of the neocortex, suggested long ago by Cajal. If memory formation implies restriction in the modes of response of the units involved, it might be of adaptive advantage for finely structured receiving and processing assemblies not to be memory forming.

The function of large-celled arrays lying on the

effector or output side of a given system may then be to "smooth out" the results of this processing, each unit acting as a computer of average transients, in which the computation is performed more spatially than serially. Such arrays may serve to convert the activity of smaller, more numerous presynaptic units into simplified resultants. In this arrangement there may be a twofold advantage.

First, presimplification of the input to systems such as the neocortex may reduce or minimize irrelevant detail, e.g., adaptively insignificant variations in otherwise standard items of perception. As a result, those complexes of sense-data which become CS's or items of recall are not literal representations of external fact. While representing certain occasions in the life history of the organism, they are themselves in the nature of generalizations, in that each datum in the complex represents the averaged effect of events occurring at the sensory periphery only. At central stations such as the neocortex, processing of inputs may include comparison of these data on a match-mismatch (summation-no summation) basis with items stored in memory, and labile combinative activities equivalent to the "hypothesis" making of Krechevsky (see Thorpe, 1963) or the "plans" of Miller, Galanter and Pribram (1960). The extent to which these supplement behavior based on Hull-Spence "continuity" learning is presumably a function of the complexity of the brain concerned, or of the complexity of the subsystem in a given brain. (It might be noted that in much rhinencephalic cortex, the external lamina is very thin, the plexus of specific afferents approaching the plexiform layer [Lorente de Nó, 1949]. Mammals lacking neocortex or left only with rhinencephalic, show radical losses in learning ability and behavioral plasticity.)

Either in such logical operations, or in simple matching processes, presimlification of the input might be thought to favor efficiency. For example, if recognition of an enemy depends upon matching a visual input with a visual memory, both input and memory need be only just particularized enough to guarantee a match under the normal range of conditions. Further particularization might only slow the matching process, or even block it as a result of variations in the input from one occasion to another.

On the effector side of the CNS, with the contraction of subsystem cell-populations and the further increase in

size of output units, the simplification process just described very likely reaches a maximum. Its function here is probably to insure that the body, as a muscular machine, can carry out the instructions issued to it from higher neuraxial levels.

The present state of the nervous system in many forms may thus represent a nice balance between the degree of sensory resolution needed for effective recognition and the degree of coarseness needed for the prompt central processing of information and the issuance of prompt practicable orders to the effector systems.

"LEARNING" vs. "NON-LEARNING" OR "LEARNING-RESISTANT" UNITS

Suppose we now make two crucial assumptions. The first is that in higher evolutionary forms, the storage of information may have moved far centrally, e.g., in mammals, to stations lying beyond the primary cortical receiving areas. That responses in striate cortex in the mature animal may be "wired in" or resistant to change through experience, is suggested by the work of Hubel and Wiesel, 1963. The second assumption is that in those central systems in which storage does occur, a certain fraction only, of the neuronal population may be involved. The original ground for this assumption is the intuitive, and possibly mistaken, notion that unless a considerable number of units were to remain, in Nauta's phrase, "uncommitted", organisms would at maturity show a rigidification of behavior far beyond that actually observed.

Taken together with the starting premise here -- that the learning of actions or outflows is a prime function of nervous systems -- these assumptions lead to the conclusions: 1) that the proportion of learning neurons may increase as one moves centrally from the receptors into major "processing" and effector divisions of the CNS, and 2) that in any given subsystem, learning units when present may be found chiefly on its output side. In support of (1), evidence just cited is against learning in the visual pathway, e.g., over the first four synapses in mature mammals, whereas learning may well occur in temporal association cortex, and in a more limited and special sense, at the level of the ventral horn (see Chamberlain, Halick and Gerard, 1963). The discussion which follows will chiefly concern (2).

Earlier it was pointed out that large-celled bands are frequently found on the output side of central subsystems, this being increasingly the case in neocortex as one moves from prime receiving to association, premotor and motor areas. If one tentatively concludes that such bands may represent the fraction of neurons in a given subsystem which are capable of learning, then the significance of several of the points made above becomes clearer.

First, Lashley's principle of economy in the recall of sensory detail might in part be attributed to the fact that the same units which simplify sense-data in transit are those most apt to "remember" it. Secondly, whereas memory functions may be minimal in koniose (prime receiving) cortex in which large-pyramidal arrays in layer V are poorly developed, a considerable body of clinical and physiological evidence implies important memory functions for parts of posterior association cortex, in which large-celled arrays in IIIc and V are commonly found (Bailey and von Bonin, 1951). Thirdly, such a segregation of memory units on the output side of n subsystems from central stations to the final effector paths would serve to give priority to the learning of outflows or actions.

Nonlearning or learning-resistant units may then be concentrated on the receptor side of the same subsystems, increasing in proportion and finally predominating, including output units, as one moves from central stations toward the peripheral receptors. Those units which are nonlearning may be so by virtue of highly restricted or specialized connections, as in the case of the striate and lateral geniculate units studied by Hubel and Wiesel, or the somatosensory units studied by Mountcastle and Powell (Mountcastle 1957; Mountcastle and Powell 1959). Having virtually no "choice" of inputs, such units may ab initio be unlikely to develop those receptive field changes postulated here to underlie memory formation, the same restriction applying to large output units in peripheral receptors such as the retina. Whether the latter statement also applies to phylogenetically older receptor systems, such as the olfactory bulb, is a question of some interest, particularly in view of the unique persistence of olfactory avoidance CR's in the monkey, reported by Pechtel and Masserman, 1954.

Learning-resistant units may differ from the foregoing nonlearning units only in respect of connectivity. That is, as result of more diverse inputs, such units may have a

greater likelihood of developing the receptive field changes equivalent to preferences for certain of these. Units of this kind are perhaps exemplified by those found in association areas, which typically show greater variability in their receptive fields, longer and more variable latencies, and greater evidence of cross-modal and/or specific-nonspecific convergence than do units in prime receiving areas (Morillo, 1961; Carreras and Anderson 1963; Thompson, Johnson and Hoopes, 1963). Such units may be chiefly responsible for the processing of inputs in secondary receiving areas of the neocortex, and perhaps occur in the greatest concentration on the input side (i.e., in the external lamina) of vertical columns. They may be distinguished from learning units lying on the output side (in the internal lamina) by the fact that, in the latter, induced receptive field changes may be more lasting by a factor of several powers of ten (see below).

In summary, the discussion thus far comes down to this -- that in the vertebrate CNS there may be two major neuronal populations. The first is composed of units in which memory formation either may not occur at all because of restriction of inputs, or may occur but reversibly. Such units may predominate at the sensory periphery, and in subsystems a few synapses from the latter may be found chiefly on the receptor or input side. In systems lying close to the effector periphery, their locus within the system will be the same but their numbers, relative to those found in more central systems and to output units in the same system, will be reduced. The second population, consisting of units in which lasting memory formation may occur, may then have approximately the converse distribution, both in the CNS as a whole and within those subsystems in which they are found at all.

Units of the first population, when their inputs are sufficiently diverse (as in association cortex) may comprise the so-called short-term memory system (see e.g., Pribram, 1959). From the clinical evidence of Scoville and Milner (1955;1957) it would appear that this system is independent of the septal-hippocampal-entorhinal complex in that it continues to function after the capacity to form lasting memories is lost. The Scoville-Milner data suggest that in man, the short-term retention span is on the order of fifteen minutes. (It is of some interest that Henry, one of Milner's patients, showed a normal learning curve for a mirror-writing task but could form no day-to-day memories of the test situation, expressing surprise at his proficiency in a "new"

task. This suggests dissociation of motor and sensory learning, perhaps within the neocortex proper. Milner, 1964).

EVIDENCE FOR HISTOCHEMICALLY DISTINCT POPULATIONS, CORRESPONDING TO THE DISTINCTION BETWEEN RECEPTOR AND EFFECTOR DIVISIONS OF CENTRAL SUBSYSTEMS

On the hypothesis adopted here, short-term memory implies the existence of a population of neurons in which structural alterations, e.g., in postsynaptic membrane, are readily reversible. This in turn implies that the metabolic turnover in such neurons must be significantly higher than it is in units in which such alterations, once established, tend to persist for hours, days or years. That is, it is assumed that while such alterations may be induced or triggered by the output of presynaptic units, the energy by which they are brought about or subsequently "reversed" is supplied by the cell itself, possibly with synergistic participation of the glia (Hydén, 1963; Adey, 1963; Fatehchand, 1964).

This line of reasoning leads to the following predictions. First, on the receptor side of central subsystems, one should find evidence of populations of cells having in general higher metabolic rates than are found among units on the effector (output) side of the same systems. Secondly, there should be evidence that this activity is concentrated in neuronal receptor membrane, since it is chiefly there that the structural alterations postulated to underlie memory formation may take place.

Vladimirov et al. (1961) assayed the laminar distribution of ATP and phosphocreatine in the motor, auditory, visual, and sensory cortices of the rat. In all cases, maximal concentrations of these substances were found in the external lamina (layers I-IV), and lower or minimal concentrations in the internal lamina (layers V-VI). Friede (1961) made assays of succinic dehydrogenase in cortical and subcortical systems in the guinea pig and cat, by a method which permitted him to determine the approximate intracellular distribution of the enzyme. In this way he defined a population of "effector" units at the base of the neocortex (layers V-VI; guinea pig) in which the enzyme was concentrated in perikaryal membrane, and a population of "receptor" units in layers I-IV in which distribution of the enzyme was more dendritic than somatic.

Similarly, in the brainstem reticular formation of the cat, he distinguished a medial magnocellular "effector" population having a primarily somatic distribution, and a laterally lying small-celled "receptor" population, having a primarily dendritic distribution, of succinic dehydrogenase. (The latter population apparently corresponds to those lateral divisions of the RF which receive spinal projections and connect reciprocally with the cerebellum.) It should be pointed out that, as dendritic membrane may amount to 90% of the surface of cortical units, (Sholl, 1956), a dendritic distribution argues a considerably higher level of succinic dehydrogenase activity than is found in units having a somatic distribution only. Findings consistent with the foregoing were made by Friede in the thalamus, strong activity being obtained "in all the primary /cortical/ sensory areas and their thalamic nuclei". Mild to weak activity was found in frontal cortex, DM and midline nuclei. "Cytochrome oxidase showed histochemical gradations among nuclei which were like those of succinic dehydrogenase."

It would thus appear that two cell populations distinguished by sharp differences in their metabolic rates may in fact exist at several neuraxial levels in the vertebrate nervous system; and individual subsystems may have the distribution predicted on theoretical grounds here.

The findings just reviewed say nothing of course, concerning the localization of memory functions in these subsystems. Direct evidence on this point is fragmentary and ambiguous. Kogan is reported to have found that during formation of a conditioned avoidance response in the cat, increased activity was recorded from the upper four layers of the cortex; whereas at criterion such apparently evoked activity spread to include the internal lamina. Doubts have been expressed as to these results (Galambos; Purpura, 1959). Such a precise localization of activity within a given column seems highly unlikely, both on cytoarchitectural grounds and in the light of the physiological evidence of Mountcastle (1957), indicating activation of units at all cortical depths in a somatosensory column within about 2 msec (or the maximal cortical synaptic delay-time estimated by Chang, 1952).

Murphy and Dusser de Barenne (1941) performed thermocoagulation unilaterally affecting the entire precentral area of arm representation in macaques (N = 2). In the animal in which coagulation spared layers V-VI, early severe motor

deficits cleared markedly in two weeks; and by two months, performance of limbs on the operated and unoperated side was not detectably different. In the animal in which coagulation involved V and to some degree the polymorph layer, "typical and long-lasting motor deficit" resulted. In view of the evident importance of neocortical motor areas to fine-finger movements (Fulton, 1949), it might be inferred that Milner's patient Henry (see pages 43-44) had retained the capacity to form lasting neocortical motor memories; and likewise that here, a part of the observed deficit in animal number 2 may have been due to the destruction of such memories. It is still not demonstrated that that was the case, the same applying to the area 4 lesion studies reviewed by Herrick (1956), and to the work of Peterson and Devine (1963) on the forced transfer of "handedness" in rats.

Another line of evidence which may be relevant here is that derived from polarization studies. Purpura and McMurtry (1965; see also Rowland, 1963) made slow potential surface recordings while also recording intracellularly from units at various depths in cat motor cortex during surface anodal and cathodal polarization of the same region. They noted that pyramidal tract (PT) units "were generally depolarized during anodal polarization and hyperpolarized during cathodal polarization of the cortical surface. Although these effects ... were also observed in non-PT cells whose cell bodies were located below 0.7-0.9 mm, opposite effects were noted in more superficially located elements. Thus anodal polarization of the cortical surface generally hyperpolarized non-PT cells in superficial regions of cortex ... whereas cathodal polarization depolarized cells in this location." Morrell (1963) reports that acquisition of a visual conditioned response in rabbits is accelerated by bilateral anodal polarization of visual cortex, and retarded by cathodal polarization of the same areas. Significant differences in performance of the CR were also observed on the day following anodal or cathodal polarization. "Comparison of performance on the day after visual anodal polarization with that on the day preceding ... polarization yields a difference (improvement!) significant at better than the 1% level of confidence." Cathodal polarization appeared to block the normal effects of training so that on days when the two were paired, it was as though no training had been given. From these reports, one might conclude that the population of cortical cells crucially involved in establishment of a conditioned "trace" may lie in the deeper layers, as proposed in this paper.

As already noted, some of the foregoing data underscore a peculiar difficulty which automatically arises if memory is in fact chiefly a function of output or final processing units. It is that selective depression or ablation of such units will yield results comparable to those of ablation of the subsystem as a whole. This objection perhaps applies least to neocortex since there, output of vertical columns may not depend wholly upon units of the internal lamina. It is therefore possible that by surface cathodal polarization, or the immunological method mentioned below, one can selectively eliminate much of the population of units supposed here to be memory-forming, without reducing the output of the vertical columns concerned to zero. In this connection it is possibly significant that the surface negative polarizing currents used by Morrell (1963) did not appear to cause gross visual impairment, although they affected visual learning and involved, by his estimate better than 50% of visual cortex bilaterally.

ROLE OF EFFECTOR FEEDBACK TO CENTRAL STATIONS IN CONSOLIDATION; AND OF FEEDBACK FROM CENTRAL STATIONS TO THE SENSORY PERIPHERY, IN ATTENTION

To conclude this part of the argument, it might be mentioned that one function of the feedback mechanisms of which examples were given earlier, may be to favor consolidation of CR's, e.g., by establishment of "contrast" in systems upstream from those in receipt of central outflows.

In that such feedback might act to restrict the items "consolidated" to a few central ones (in the sense of central to the organism's perceptual fields), it would be a mechanism conducive to economy in recall. In that it would also become operative to the extent that any given set of inputs had resulted in action (outflows reaching the final effector paths) it would, like the recording of memories in output-arrays, favor the recall of behavior, or more precisely, of sense-data in proportion as receipt of these was succeeded by actions. As a result of this arrangement there would tend, in general, to be what one could describe as functional consistency between data recorded at central stations and in systems lying closer to or at the effector periphery. Conversely, those sense-data which have not, with suitable transforms, given rise to incipient "motor" memories, may themselves have a significantly smaller probability of becoming permanent items of recall. That this may in fact be

the case is suggested by the animal studies of Held and Hein (1962) and by similar studies made in man (Held and Hein, 1963; see also Berelson and Steiner, 1964; Adams, 1964).

The evidence cited by Berelson and Steiner indicates that such feedback effects may also be nonspecific, in the sense that a concurrent motor outflow may act to "consolidate" sense-data not functionally related to it. That feedback of this type may not convey information, but may merely potentiate the recording of sensory information which happens to be incoming at the time, might be inferred from the work of Girden and Culler on autonomic and skeletal motor conditioning under curare (see Morgan, 1951). Learning presumed to occur only at lower neuraxial levels under curare was not later transferred, by feedback, to higher levels; and with the return of normal hierarchical control, may have been inhibited from those levels, resulting in disappearance of the learned behavior. It also appears that normally established CR's can evidently not be triggered from some intermediate level, when function at the highest levels is impaired by curare or the spreading depression of Leão. With these qualifications, the "reafference" mechanism just described may figure in learning and amount to a form of "backward consolidation".

Within the neocortex, a mechanism of this kind, dependent upon playback from temporal-entorhinal cortex to other areas including or encroaching upon the prime receptor, seems to have been demonstrated in mice by Flexner, Flexner and Stellar (1963). Their work suggests that consolidation occurs first in temporal-entorhinal cortex (in agreement with Scoville and Milner's findings in man), and 5 to 11 days later, involves much larger cortical territories. These data imply a two-stage scheme in which the primary traces may be laid down as a result of subcortical (septal-hippocampal) influence, in a limited area. By relay from that area corresponding items in other cortical loci may then be consolidated by a process which is slower, possibly because dependent upon reinforcement (repetition of the input) alone. Transmitters may here play a negligible role. The Scoville-Milner data indicate that "fixing" of the memory in one cortical sector, e.g., as result of septal-hippocampal outflows (see Adey, 1961), may be the essential first step since, when it is prevented, the second, at least in man, cannot occur.

The notion of backward consolidation implies that conditioning proceeds in two fundamental stages -- the first

involving establishment of a path from the sensory to the effector periphery, and the second involving consolidation via feedback over long and short loops throughout the length of the path. These stages will presumably overlap in time, the second developing acceleratively as the first goes to completion. In fact, a model having these basic features has been proposed by Sutherland (1964) to account for three paradoxical properties of conditioned responses. In his model, attachment of the analyzer to the response goes more rapidly to completion than does attachment of the stimulus to the analyzer. That is, the animal more quickly fixes a learned action than it fixes a memory of the corresponding cue(s). Sutherland showed that the reversal learning curves of "stat rats" (computer-simulated behavior based on his model) and of actual rats were quite similar.

One might note, in conclusion, that the mechanisms involved in "backward consolidation" may in part be the same as those involved in establishment of attentional foci. Given an input which includes learned, or adaptively significant, components, feedback from central stations such as temporal association cortex to cortical prime receiving areas may act to select those components from a matrix of less relevant sense-data. How far toward the periphery this selective process extends in higher vertebrates is not known and deserves further study, particularly in the light of the findings recently reported by McGill (1964). It is interesting in this connection that Cajal (1909) reported that the supposed centrifugal fibers in the mammalian retina were very difficult to stain, and less prominent (or in his words "moins epaisses") than those in the avian retina.

SUGGESTED LINE OF INQUIRY FOR TESTING THE CONCLUSION THAT OUTPUT UNITS IN CENTRAL SUBSYSTEMS MAY BE THOSE CHIEFLY CONCERNED IN LEARNING

The important question for this discussion is whether or not output units corresponding to Friede's "effector" populations, e.g., in neocortex, can be shown to be chiefly or solely responsible for learning. The most obvious way to approach this problem might be by selective lesioning of cortex of the temporal-entorhinal system, bilaterally. A method is required which would enable the investigator to eliminate cells of layers V-VI of this cortex over a considerable area, with minimal damage to units of the overlying layers

or to their fiber connections with the white matter.

Admittedly, such a line of investigation presents great difficulties. Because of the last-mentioned requirement, ablations via the proton beam technique used by Malis et al. (1960) or via the focal desiccation-lesioning described by Peterson and Devine (1963) appear to be ruled out, except perhaps as ancillary approaches.

If Flexner et al. (1963) have correctly interpreted their findings, the hypothesis presented here suggests that bilateral cathodal polarization of temporal cortex in mice might mimic the effects of puromycin in their experiments. Conversely, surface anodal polarization should mimic the effects of learning-accelerators such as strychnine, or decrease the minimal time for "irradiation" of the trace from temporal to other cortical areas. The effects of surface cathodal polarization should be crudely duplicable by multiple focal lesions (see Peterson and Devine, 1963) bilaterally affecting the internal lamina; whereas the same density of lesioning, confined to layers I-IV, should interfere significantly less with learning. In any such experiment it would also be necessary to use suitable controls indicating that changes in learning were probably not attributable to changes, e.g., in the animal's "motivation" or day-to-day drive-levels.

Another possible approach is suggested by the work of Levi-Montalcini (1961) and of Mihailović (1964). Briefly, if the data of Friede (1961) and of Vladimirov et al. (1961) reflect differences also in the protein constituents of units of the internal and external laminae, it is conceivable, that antibodies selective for units of either of these populations might be developed. This method (called to the writer's attention by Oscar Hechter) is attractive in principle, but much further work will be required to determine whether it can in fact be used for the sort of selective ablation discussed here. Certain data (Galambos, 1965) suggest that possibly it cannot.

The foregoing are intended less as definite experimental proposals than as illustrations of the type of inquiry which might answer certain of the questions raised earlier in this paper.

CONCLUDING REMARKS

The increase in short axon cells or "granules" in the nervous systems of "higher" forms has been correlated with the evolutionary increase in intelligence, (Young, 1964); and memory functions have been tentatively attributed to these cells by some (see for instance, Hainer et al., 1954). However, it is suggested above that such cells may figure in the "processing", but not in the recall of sense-data. The fact that some cells of this type (e.g., retinal amacrines, or those found in the octopus optic lobe) are without axons, and that small units in general (10μ or less in diameter; see Bullock, 1959a) are not definitely known to produce action potentials, is perhaps consistent with the views outlined here. In those parts of the CNS in which the proportion of slow potential to unitary activity is highest, the probability of stable path formation may, in other words, be lowest.

The question of how much interplay or "useful noise" (Fogel, 1961) is permitted in the nervous system as a result of random connections is important and seems still to be in dispute. Whereas for instance Sholl (1956) describes stochastic growth patterns for dendritic trees in striate cortex, another investigator, Colonnier, looking at essentially the same material, sees clear morphological types (Colonnier, unpublished material. See Young, 1964). The most recently reported work of Hubel and Wiesel (1965) appears to support Colonnier in that it implies a high degree of specificity of connections in visual areas 17, 18 and 19 in the cat.

Apropos of the functions of large output units, discussed earlier, it is interesting that Green (1964) has remarked: "Any theory of memory should explain why it is a generalization that is retained rather than a response to a specific stimulus". The hypothetical memory-units proposed here correspond approximately to Bullock's "decision-making" neurons (1961). In regard to the process by which unconditioned stimuli may act to "fix" a precurrent stimulus in memory, it is well to note that not all UCS's have this property. Such differences may well relate to the organization of subcortical "motivational" systems and to the possibility, suggested by Brodie and Shore, that such systems may have characteristic transmitters. (See Fair, loc.cit. p. 181 and passim.)

If learning units tend to be concentrated on the output side of central subsystems, and if (predominantly negative or inhibitory) feedback plays a major role in consolidation and "informed" attention, as proposed here, certain correlates of the learning process appear to become more readily explainable. To the extent that any array of learning units has learned, it will, given a normal run of inputs, tend to be more promptly brought into action than was the case prior to learning. The resulting feedback from that level and from lower neuraxial levels will exert an inhibitory damping action on that and other central subsystems. Conversely, unfamiliar inputs (those with minimal correspondences in the organism's memory) will not as promptly result in central outflows, with consequent prolongation of the evoked central excitatory state. This scheme corresponds approximately to the sequence of electrocortical events often seen during conditioning -- the initial stages of which tend to be accompanied by generalized desynchronization, and the final stages, by focal activation only (Gastaut, 1958). In other words, one function of memory is to reduce the outlay of central nervous energy involved in adaptive behavior. For this reason perhaps, is is of advantage for memories themselves to have the generalized character described above, since the economy in energy-expenditure which they effect is proportioned to the range of external events which they permit the organism promptly to recognize.

SUMMARY

An hypothesis as to learning in single units (neurons) is proposed. An analysis of certain organizational features, found in many parts of the vertebrate nervous system, follows. The conclusions from that analysis are: 1) that feedback may figure in the consolidation of conditioned responses and in the establishment of attentional foci, the mechanisms involved being essentially the same in either case; and 2) that "learning" units, when present in a given subsystem, may chiefly be found on its output side. The prediction from (2) is that destruction, e.g., by immunological methods, of cells of the internal lamina bilaterally, in temporal-entorhinal cortex, will impair learning-capacity, roughly in proportion to the amount of cortex affected.

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